

S/N 09/565,041

*Amendment & Response*

66. (New) The product of claim 65, wherein the collagen of at least one of the porous layer and of the collagen membrane is selected from the group consisting of collagen and a mixture of collagen with at least one polysaccharide selected from the group consisting of a cellulose, a dextran, an alginate, a carrageenan, a glycosaminoglycan, and a chitosan.

67. (New) A composite product comprising the composite acellular product of claim 65, wherein at least one of the porous layer and of the collagen membrane, comprises living cells selected from the group consisting of normal living cells, genetically modified living cells and malignant living cells.

68. (New) The product of claim 67, wherein said living cells originate from young subjects.

69. (New) The product of claim 67, wherein said living cells originate from elderly subjects.

70. (New) The product of claim 67, wherein the living cells are selected from the group consisting of fibroblasts, keratinocytes, melanocytes, Langerhans' cells originating from the blood, endothelial cells originating from the blood, blood cells, sebocytes, chondrocytes, osteocytes, osteoblasts, nervous cells and Merkel's cells, said cells being normal, genetically modified or malignant.

71. (New) A composite skin product forming a collagen support comprising at least one porous collagen layer covered on at least one side with a collagen membrane prepared by a compression of a collagen sponge at a pressure of at least about 50 bar, said porous collagen layer comprising living fibroblasts and said collagen membrane comprising on the surface thereof living cells selected from the group consisting of: keratinocytes, melanocytes, nervous cells, Merkel's cells, Langerhans' cells originating from the blood, sebocytes, and cells originating from the blood.

S/N 09/565,041

*Amendment & Response*

72. (New) The product of claim 71, wherein the collagen sponge is compressed at a pressure of at least about 50 bar, and at a temperature ranging between about 20°C and 80°C.

Sub 83  
73. (New) The product of claim 71, wherein the collagen sponge is compressed at the pressure ranging between about 50 bar and 200 bar and at a temperature between about 40°C and 60°C.

74. (New) The product of claim 70, wherein the collagen membrane is prepared prior to combination with the porous layer.

Sub 83  
DI  
75. (New) The product of claim 70, wherein after having prepared the membrane, a collagen gel is deposited on at least one surface of the membrane and the combination of the collagen gel with the membrane is frozen and lyophilized to give said composite product.

DI  
76. (New) A composite acellular product forming a collagen support comprising at least one porous collagen layer covered on at least one side with a collagen membrane prepared by a compression of a collagen sponge at a pressure of at least about 50 bar, and wherein at least one of the porous layer and membrane is produced from a collagen gel containing a mixture of soluble collagen and insoluble collagen.

77. (New) The product of claim 76, wherein said insoluble collagen comprises collagen fibers.

Sub 84  
78. (New) The product of claim 65, wherein at least one of the porous layer and collagen membrane is produced from a collagen gel containing a mixture of soluble collagen and insoluble collagen, wherein the collagen is selected from the group consisting of type I collagen and type III collagen.

79. (New) An artificial skin comprising a product as defined in claim 65, 67, 71, or 76.

S/N 09/565,041

*Amendment & Response*

80. (New) The artificial skin of claim 79, comprising living cells obtained from young subjects.

81. (New) The artificial skin of claim 79, wherein said artificial skin comprises living cells obtained from elderly subjects.

Sub  
E1  
82 (New) The artificial skin of claim 79, comprising living cells selected from the group consisting of fibroblasts, keratinocytes, melanocytes, Langerhans' cells originating from the blood, endothelial cells originating from the blood, blood cells, sebocytes, nervous cells, chondrocytes, osteocytes, osteoblasts and Merkel's cells, said cells being normal, genetically modified or malignant.

DI  
Cont.  
83. (New) The artificial skin of claim 82, wherein said blood cells are selected from the group consisting of macrophages and lymphocytes.

Sub  
E6  
84. (New) The artificial skin of claim 80, wherein at least one of the porous layer and of the collagen membrane comprises a compound which favors cell development.

85. (New) The artificial skin of claim 84, wherein said compound which favors cell development is selected from the group consisting of a growth factor, cytokine and a chemokine.

Sub  
E7  
86. (New) The artificial skin of claim 79, wherein collagen is selected from the group consisting of collagen and a mixture of collagen with at least one polysaccharide selected from the group consisting of a cellulose, a dextran, an alginate, a carrageenan, a glycosaminoglycan, and a chitosan.

87. (New) The artificial skin of claim 79, wherein at least one of the porous layer and of the collagen membrane is crosslinked.

S/N 09/565,041

*Amendment & Response*

88. (New) The artificial skin of claim 87, wherein crosslinking is a thermal dehydration under vacuum.

89. (New) The artificial skin of claim 87, wherein crosslinking is a chemical crosslinking selected from the group consisting of a crosslinking with diphenyl phosphorylaxide, crosslinking with an aldehyde, crosslinking with glutaraldehyde, a crosslinking with a carbodiimide, a crosslinking with a succinimide and combinations thereof.

90. (New) A method of reconstructing damaged areas of skin in vivo comprising performing said reconstruction with an artificial skin prepared from a product selected from the group consisting of a composite product as defined in claim 67, 71, 76, or 79.

91. (New) The method of claim 90, wherein at least one of the two layers is produced from a collagen gel containing a mixture of soluble collagen and insoluble collagen, the collagen being selected from the group consisting of type I collagen and Type III collagen.

92. (New) The method of claim 90, wherein said artificial skin comprises living cells obtained from young subjects.

93. (New) The method of claim 90, wherein said artificial skin comprises living cells obtained from elderly subjects.

94. (New) The method of claim 90, comprising living cells selected from the group consisting of fibroblasts, keratinocytes, melanocytes, Langerhans' cells originating from the blood, endothelial cells originating from the blood, blood cells, sebocytes, chondrocytes, osteocytes, osteoblasts, nervous cells and Merkel's cells, said cells being normal, genetically modified or malignant.

95. (New) The method of claim 94, wherein said blood cells are selected from the group consisting of macrophages and lymphocytes.

S/N 09/565,041

*Amendment & Response*

96. (New) A method of in vitro testing of the efficacy of a potentially active substance comprising monitoring the effect of said potentially active substance on an artificial skin prepared from a composite product as defined in claim 67, 71, 76, or 79, wherein said artificial skin comprises living cells obtained from young subjects.

97. (New) A method of in vitro testing of the efficacy of a potentially active substance comprising monitoring the effect of said potentially active substance on an artificial skin prepared from a composite product as defined in claim 67, 71, 76, or 79, wherein said artificial skin comprises living cells obtained from young subjects.

98. (New) A composite acellular product forming a collagen support comprising at least one porous collagen layer covered on at least one side with a collagen membrane comprising a collagen film prepared by drying a collagen gel separately from the porous collagen layer.

99. (New) The product of claim 98, wherein the collagen of at least one of the porous layer and of the collagen membrane is selected from the group consisting of collagen and a mixture of collagen with at least one polysaccharide selected from the group consisting of a cellulose, a dextran, an alginate, a carrageenan, a glycosaminoglycan, and a chitosan.

100. (New) A composite product comprising the composite acellular product of claim 98, wherein at least one of the porous layer and of the collagen membrane, comprises living cells selected from the group consisting of normal living cells, genetically modified living cells and malignant living cells.

101. (New) The product of claim 100, wherein said living cells originate from young subjects.

S/N 09/565,041

*Amendment & Response*

102. (New) The product of claim 100, wherein said living cells originate from elderly subjects.

Sub  
EQ  
103. (New) The product of claim 100, wherein the living cells are selected from the group consisting of fibroblasts, keratinocytes, melanocytes, Langerhans' cells originating from the blood, endothelial cells originating from the blood, blood cells, sebocytes, chondrocytes, osteocytes, osteoblasts nervous cells and Merkel's cells, said cells being normal, genetically modified or malignant.

D  
Coul.  
104. (New) An artificial skin product forming a collagen support comprising at least one porous collagen layer covered on at least one side with a collagen membrane prepared by drying a collagen gel separately from the porous collagen layer, said porous collagen layer comprising living fibroblasts and said compact membrane comprising on the surface thereof living cells selected from the group consisting of: keratinocytes, melanocytes, nervous cells, Merkel's cells, Langerhans' cells originating from the blood, and sebocytes.

105. (New) The product of claim 104, wherein the collagen membrane is prepared by drying the collagen gel in air.

Sub  
Coul.  
106. (New) The product of claim 105, wherein after having prepared the membrane, a collagen gel is deposited on at least one surface of the membrane and the combination of the collagen gel with the membrane is frozen and lyophilized to give said composite product.

107. (New) A composite acellular product forming a collagen support comprising at least one porous collagen layer covered on at least one side with a collagen membrane prepared by drying a collagen gel separately from the porous collagen layer, and wherein at least one of the porous layer and of the collagen membrane is produced from a collagen gel containing a mixture of soluble collagen and insoluble collagen.

S/N 09/565,041

*Amendment & Response*

108. (New) The product of claim 107, wherein said insoluble collagen comprises collagen fibers.

109. (New) The product of claim 98, wherein at least one of the porous layer and of the collagen membrane is produced from a collagen gel containing a mixture of soluble collagen and insoluble collagen, wherein the collagen is selected from the group consisting of type I collagen and type III collagen.

110. (New) An artificial skin comprising a product selected from a product as defined in claim 98, 100, 104, or 107.

111. (New) The artificial skin of claim 110, comprising living cells obtained from young subjects.

112. (New) The artificial skin of claim 110, comprising living cells obtained from elderly patients.

113. (New) The artificial skin of claim 110, comprising living cells selected from the group consisting of fibroblasts, keratinocytes, melanocytes, Langerhans' cells originating from the blood, endothelial cells originating from the blood, blood cells, sebocytes, chondrocytes, osteocytes, osteoblasts, nervous cells and Merkel's cells, said cells being normal, genetically modified or malignant.

114. (New) The artificial skin of claim 113, wherein said blood cells are selected from the group consisting of macrophages and lymphocytes.

115. (New) The artificial skin of claim 110, wherein at least one of the porous layer and of the collagen membrane comprises a compound which favors cell development.

116. (New) The artificial skin of claim 115, wherein said compound which favors cell development is selected from the group consisting of a growth factor, cytokine and a chemokine

S/N 09/565,041

*Amendment & Response*

117. (New) The artificial skin of claim 110, wherein the collagen is selected from the group consisting of a collagen and a mixture of collagen with one substance at least selected from the group consisting of at least one selected from the group consisting of cellulose, dextran, alginate, carrageenan, sulfated glycosaminoglycans, and chitosan.

118. (New) The artificial skin of claim 110, wherein at least one of the porous layer and of the collagen membrane is crosslinked.

119. (New) The artificial skin of claim 118, wherein said crosslinking is a thermal dehydration under vacuum.

120. (New) The artificial skin of claim 118, wherein crosslinking is a chemical crosslinking selected from the group consisting of a crosslinking with diphenyl phosphorylazide, crosslinking with an aldehyde, crosslinking with glutaraldehyde, a crosslinking with a carbodiimide, a crosslinking with a succinimide and combinations thereof.

121. (New) An artificial skin product forming a collagen support comprising at least one porous collagen layer covered on at least one side with a collagen membrane comprising a collagen film prepared by drying a collagen gel in air separately from the porous collagen layer, said porous collagen layer comprising living fibroblasts cells and said collagen membrane comprising on the surface thereof living cells other than fibroblasts.

122. (New) The product of claim 121, wherein said living cells on the surface of the membrane are selected from the group consisting of keratinocytes, melanocytes, nervous cells, Merkel's cells, Langerhans' cells originating from the blood, sebocytes, and cells originating from the blood.

123. (New) The product of claim 121, wherein said living cells on the surface of the membrane comprise keratinocytes.



S/N 09/565,041

*Amendment & Response*

124. (New) A method of reconstructing damaged areas of skin in vivo comprising performing said reconstruction with an artificial skin prepared from a product selected from the group consisting of a composite product as defined in claim 100, 104, 107, or 121.

125. (New) The method of claim 124, wherein at least one of the two layers is produced from a collagen gel containing a mixture of soluble collagen and insoluble collagen, the collagen being selected from the group consisting of type I collagen and Type III collagen.

126. (New) The method of claim 124, wherein said artificial skin comprises living cells obtained from young subjects.

DI  
cost. 127. (Amended) The method of claim 124, wherein said artificial skin comprises living cells obtained from elderly subjects.

128. (Amended) A method of in vitro testing of the efficacy of a potentially active substance comprising monitoring the effect of said potentially active substance on an artificial skin prepared from a composite product as defined in claim 100, 104, or 121, wherein said artificial skin comprises living cells obtained from young subjects.

129. (Amended) A method of in vitro testing of the efficacy of a potentially active substance comprising monitoring the effect of said potentially active substance on an artificial skin prepared from a composite product as defined in claim 100, 104, 107, or 121, wherein said artificial skin comprises living cells obtained from elderly subjects.

sub  
sub 130. (New) The product of claim 71, wherein the product is cultivated in a compatible culture medium with the surface layer comprising the living cells emerged at the air-liquid interface and the porous layer comprising the fibroblasts immersed, and wherein said cultivation results in a reconstructed skin composed of a reconstructed dermis, comprising the fibroblasts

S/N 09 565,041

*Amendment & Response*

that colonized the porous collagen layer forming a three dimensional matrix, said dermis being covered with a multilayer epidermis comprising said collagen membrane.

5.6  
3.5  
3.0  
D!  
cact.

131. (New) The product of claim 104, wherein the product is cultivated in a compatible culture medium with the surface layer comprising the living cells emerged at the air-liquid interface and the porous layer comprising the fibroblasts immersed, and wherein said cultivation results in a reconstructed skin composed of a reconstructed dermis, comprising the fibroblasts that colonized the porous collagen layer forming a three dimensional matrix, said dermis being covered with a multilayer epidermis comprising said collagen membrane.

132. (New) The product of claim 121, wherein the product is cultivated in a compatible culture medium with the surface layer comprising the living cells emerged at the air-liquid interface and the porous layer comprising the fibroblasts immersed, and wherein said cultivation results in a reconstructed skin composed of a reconstructed dermis, comprising the fibroblasts that colonized the porous collagen layer forming a three dimensional matrix, said dermis being covered with a multilayer epidermis comprising said collagen membrane.

---